4 ANSWER 4 OF 9 MEDLINE
AN 96353396 MEDLINE
DN 96353396
TI **Retroviral** transfer of the multidrug resistance-1 **gene** into lineage-committed and primitive hemopoietic cells.
AU Fruehauf S; Boesen J J; Breems D A; Hoft P; Hundsdorfer P; Zeller W J; Lowenberg B; Ploemacher R E; Haas R; Valerio D
CS Department of Internal Medicine V, University of Heidelberg, Germany.
SO STEM CELLS, (1995 Dec) 13 Suppl 3 83-9. Ref: 43
Journal code: BN2. ISSN: 1066-5099.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199612
AB Transfer of the multidrug resistance-1 (MDR1) **gene** to hemopoietic cells for myeloprotection against cytostatic agents is a new and rapidly developing field in "cancer **gene therapy**." Before clinical application, safety and efficacy criteria need to be met. The **retroviral producer cell** lines and the **retroviral** supernatant need to be tested for replication-competent **retrovirus** and contamination with adventitious agents. The **cell** source needs to contain sufficient hemopoietic cells with repopulating ability. We used CD34(+)-selected mobilized peripheral blood progenitor cells (PBPC) for MDR1 transductions in order to obtain a favorable vector to target **cell** ratio. An analysis of 249 patients who had undergone PBPC harvesting revealed that primarily solid tumor and non-Hodgkin's lymphoma patients are eligible for CD34+ selection. They can be expected to retain sufficient CD34+ cells for rapid and sustained engraftment after myeloablative **therapy** if the CD34+ **cell** loss (approximately 50%) during the procedure is taken into account. Clinical MDR1 **gene therapy** protocols focus on these two **patient** groups. Next we characterized MDR1 **gene** transfer into lineage-committed and primitive hemopoietic cells. Provirus-specific polymerase chain reactions showed a high efficiency **gene** transfer into colony-forming-units granulocyte-macrophage and long-term culture cells. The level of the conferred P-glycoprotein expression was estimated by fluorescence-activated **cell** sorting analysis to be up to 3 log above mock-transduced controls. The cobblestone area forming **cell** assay, which is a stroma-dependent long-term culture assay measuring frequencies of stem **cell** subsets in a limiting-dilution set-up, allowed demonstration of sustained expression of the MDR1 **gene** in the progeny of primitive hemopoietic cells. This is a favorable basis for a clinical MDR1 **gene therapy** trial.
CT Check Tags: Human; Support, Non-U.S. Gov't
***Drug Resistance, Multiple: GE, genetics**
Drug Resistance, Neoplasm: GE, genetics
*Hematopoietic Stem Cells: PH, physiology
Hematopoietic Stem Cells: VI, virology
Retroviridae: GE, genetics

4 ANSWER 5 OF 9 MEDLINE
AN 96159131 MEDLINE
DN 96159131
TI Analysis of trans-dominant mutants of the HIV type 1 Rev protein for their
ability to inhibit Rev function, HIV type 1 replication, and their use as anti-HIV **gene therapeutics**.
AU Ragheb J A; Bressler P; Daucher M; Chiang L; Chuan M K; Vandendriessche T;
Morgan R A
CS National Heart, Lung, and Blood Institute, Bethesda, Maryland, USA.
SO AIDS RESEARCH AND HUMAN RETROVIRUSES, (1995 Nov) 11 (11) 1343-53.
Journal code: ART. ISSN: 0889-2229.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199605
AB The HIV-1 rev **gene** product facilitates the transport of singly spliced and unspliced HIV-1 transcripts and is necessary for productive HIV-1 infection. On the basis of the previously described trans-dominant Rev mutant M10, four point mutants and one frameshift mutant of the Rev protein were constructed. The mutants were inserted into **retroviral** expression vectors and analyzed for their ability to inhibit Rev-mediated **gene** expression. Transient transfection systems were used to screen these new mutants, and each was shown to inhibit expression of a Rev-dependent CAT reporter plasmid. Inhibition of HIV-1 envelope **gene** expression was tested in the HeLa-T4 **cell** line and was also shown to be inhibited by the trans-dominant Rev mutants. **Retroviral** vector **producer cell** lines were constructed and used to transduce Rev trans-dominant **genes** into the human T-**cell** line SupT1. The engineered SupT1 **cell** lines were then challenged with HIV-1 IIIB and HIV-1 expression was monitored by Northern blot analysis and in situ hybridization. SupT1 cells expressing either a Rev point mutant or the frameshift mutant showed greatly reduced HIV-1 mRNA accumulation and the Rev-dependent singly spliced and unspliced HIV-1 mRNAs were reduced. The kinetics of viral replication following challenge of Rev trans-dominant-engineered SupT1 cells with both HIV-1 IIIB and MN strains was significantly reduced and cells were protected from viral lysis. Viruses that emerge late in infection from Rev trans-dominant-engineered cultures are not resistant to Rev-mediated inhibition. Last, trans-dominant Rev-mediated protection of human CD4+ lymphocytes from challenge with primary HIV-1 **patient** isolates confirms the potential utility of this system as an anti-HIV-1 **gene therapy** approach.
MT Check Tags: Animal; Human; Support, Non-U.S. Gov't
Amino Acid Sequence
Base Sequence
Cell Line
Chloramphenicol O-Acetyltransferase: GE, genetics
Frameshift Mutation
Gene Products, env: GE, genetics
***Gene Products, rev: GE, genetics**
Gene Products, rev: TU, therapeutic use
Gene Therapy
Genes, Dominant
Genes, Reporter
***HIV Infections: TH, therapy**

HIV Infections: VI, virology
***HIV-1: GE, genetics**
HIV-1: PH, physiology
Molecular Sequence Data
*Mutation
Point Mutation
RNA, Messenger: ME, metabolism
RNA, Viral: ME, metabolism
Transfection
Virus Replication: PH, physiology
CN EC 2.3.1.28 (Chloramphenicol O-Acetyltransferase); 0 (**Gene** Products, env); 0 (**Gene** Product, rev); 0 (RNA, Messenger); 0 (RNA, Viral)

L4 ANSWER 6 OF 9 MEDLINE
AN 96039374 MEDLINE
DN 96039374
TI Transduction of CD34-enriched human peripheral and umbilical cord blood progenitors using a **retroviral** vector with the Fanconi anemia group C **gene**.
AU Walsh C E; Mann N M; Emmons R V; Wang S; Liu J M
CS Clinical Pathology Department, NIH, Bethesda, MD, USA..
SO JOURNAL OF INVESTIGATIVE MEDICINE, (1995 Aug) 43 (4) 379-85.
Journal code: B8X. ISSN: 1081-5589.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
EM 199601
AB BACKGROUND: Fanconi anemia (FA) is an autosomal recessive inherited form of bone marrow failure. FA cells are characterized by their extreme sensitivity to DNA cross-linking agents that cause DNA instability and **cell** death. Four **genetic** complementation groups for FA have been identified and the **gene** for the complementation C group (FACC) has been cloned. **Genetic** transfer of the FACC **gene** should provide a growth advantage in transduced hematopoietic cells. We have previously demonstrated efficient **retroviral** -mediated **gene** transduction and correction of FA(C) **cell** lines and peripheral blood-derived CD34+ progenitors from patients carrying mutant FACC alleles. In this report we sought to define the optimal conditions for transduction of CD34+ progenitors from mobilized peripheral blood and umbilical cord blood. METHODS: Peripheral blood hematopoietic progenitors were obtained by G-CSF mobilization followed by apheresis. Human fetal cord blood cells were obtained from full-term gestation deliveries. Cells were immunoselected for CD34 antigen expression and then incubated with recombinant **retroviruses** containing a selectable marker **gene** (neomycin). Recombinant colony stimulating factors were added to facilitate viral transduction. Cells were plated in methylcellulose and resulting hematopoietic colonies were isolated and analyzed by PCR. RESULTS: Transduction efficiency of peripheral blood progenitors (from normal individuals) using a **retrovirus** encoding the FACC cDNA was comparable to that of the **retroviral producer** cDNA.4D currently being used in clinical **gene therapy** marking studies. We extended our standard transduction protocol to analyze CD34+ and CD34+ CD38-subpopulations of progenitors derived from umbilical cord blood (from normal pregnancies). In addition, we tested whether FACC cDNA transduction could be improved by vector infection supported by autologous stroma. For FA(C) hematopoietic **cell** infection, vector supernatant [illegible] in the presence of recombinant human IL-3, IL-6, and SCF was

patient
retroviral
[illegible] **gene therapy** [illegible]

utilizing progenitor cells from both peripheral blood and umbilical cord blood.

CT Check Tags: Human
***Antigens, CD34: GE, genetics**
Base Sequence
***Fanconi's Anemia: GE, genetics**
Fetal Blood
***Gene Transfer**
***Genetic Vectors: GE, genetics**
*Hematopoietic Stem Cells
Hematopoietic Stem Cells: IM, immunology
Molecular Sequence Data
***Retroviridae**
***Transduction, Genetic**
CN 0 (Antigens, CD34); 0 (**Genetic** Vectors)

L4 ANSWER 9 OF 9 MEDLINE
AN 89293182 MEDLINE
DN 89293182
TI **Gene** transfer into primates and prospects for **gene** **therapy** in humans.
AU Cornetta K; Wieder R; Anderson W F
SO PROGRESS IN NUCLEIC ACID RESEARCH AND MOLECULAR BIOLOGY, (1989) 36 311-22.
Ref: 24
Journal code: Q4X. ISSN: 1079-6603.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW LITERATURE)
LA English
EM 198909
AB **Retroviral** vectors infect primate bone marrow cells and express in vivo the transferred **genes** (the human ADA **gene** and the bacterial **gene** for neomycin resistance). The SAX vector appears to express human ADA at normal levels, but the infection efficiency is low (less than 1%) so that the **gene** product is only detectable in the peripheral blood at low levels. Vector expression disappears after 5 months (except for occasional T cells), presumably due to a failure to infect a renewal stem **cell**. While the level of ADA expression obtained in primates would not appear to be sufficient to correct outright the disease caused by ADA deficiency, it is possible that T-**cell** progenitors in the marrow will have a selective advantage. T cells expressing an ADA vector would then able to expand and potentially restore immune function. Unfortunately, this hypothesis will go untested until an animal model for ADA deficiency is found or a human clinical trial is performed. At present, consideration of **gene** **therapy** as a treatment for ADA deficiency would only be appropriate if all conventional forms of treatment were unsuccessful. If such a scenario should present itself, the critical question becomes one of safety, to both the **patient** and those in contact with the **patient**. We have begun to address the safety issues associated with **gene** **therapy**. Five animals exposed to replication-competent **retrovirus** during bone marrow transplantation show no evidence of helper virus, with a mean follow-up of 18.3 months. Four animals injected with replication-competent helper virus cleared the virus rapidly and, after the initial clearance, have shown no evidence of **retroviremia**, with a mean follow-up of 5.2 months. Our preliminary findings suggest that murine retroviruses do not cause a productive infection in vivo. These results, combined with the availability of better **producer cell** lines free of helper virus, are encouraging, and suggest that the risk of clinical disease from murine **retrovirus** introduced by a **gene** **therapy** protocol should be small. Unfortunately, high infection efficiency and long-term vector expression still must be obtained before **retroviral**-mediated **gene** transfer can be considered as first-line **therapy** for ADA deficiency.

*Gene Therapy
Genetic Vectors

*Primates: GE, genetics
Retroviridae: GE, genetics
*Transfection

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      (FILE 'HOME' ENTERED AT 12:37:54 ON 15 MAR 2000)

      FILE 'MEDLINE' ENTERED AT 12:38:52 ON 15 MAR 2000
L1         2527 S RETROVIR?
L2          3000 S L1 AND (GENE? AND THERAP?)
L3           126 S L2 AND (PRODUCER AND CELL)
L4             9 S L3 AND PATIENT

      FILE 'CAPLUS' ENTERED AT 12:45:35 ON 15 MAR 2000
L5         16730 S L1
L6          3162 S L2
L7           162 S L3
L8             9 S L4
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